IMMUNOCHEMISTRY*

By Michael Heidelberger

*Department of Medicine, College of Physicians and Surgeons
of Columbia University, and the Presbyterian Hospital,
New York City*

As in last year's review, papers which do not deal in some way with the chemical basis of immunology have been excluded. Space requirements have made it necessary to eliminate many others of undoubted interest. The period covered is essentially that from December, 1931, to November, 1932, and the subject is treated under two main headings: I. The Chemistry of Immune Substances; II. The Chemistry of Immune Reactions.

I. THE CHEMISTRY OF IMMUNE SUBSTANCES

1. Naturally Occurring Proteins and Protein Derivatives as Antigens

Fowl livetin has been separated from egg yolks by Jukes & Kay (1). In anaphylaxis and precipitin tests it showed a close relationship or identity with fowl-serum globulins. According to Proca (2), beef-choroid melanin showed antigenic properties only in albino rabbits, yielding sera giving complement fixation with beef, pig, sheep, and chicken melanin. Rabbit melanin also produced similar antibodies in albino rabbits. It is interesting in this connection that Waelsch (3) found that the pigment fraction of melanin is firmly linked to a protein of distinctive properties.

Kirk & Sumner (4) have shown that jack bean urease antiserum inactivates soy bean urease and protects rabbits against soy urease, so that the enzyme appears to be identical in the two species of bean.

Among the bacterial proteins, an interesting product has been extracted with acid alcohol from S- and R-forms of the *Salmonella* group by P. B. White (5). The protein is insoluble in neutral or alkaline alcohol and soluble in dilute alkalies or hydrochloric acid. Its solutions are precipitated by salts and give typical protein reactions. It is antigenic, yielding precipitating sera which agglutinate S-forms least readily. The same product appears to be present in the coliform,

dysentery, and proteus groups. Its removal interferes with somatic agglutination, possibly owing at least in part to its ready salt-precipitability. In the same group, Olitzki (6) has found it possible by cataphoresis of *Proteus X* 19 extracts at pH 4 to obtain the "O" antigen at the positive compartment, leaving the "H" antigen in the middle vessel.

As regards tubercle bacillus proteins, Seibert & Munday (7) have carried still further the purification of unheated tuberculin, finding that the active protein may be freed from almost all accompanying polysaccharide by precipitating and washing with trichloroacetic acid. Seibert (8) has also shown that if sufficiently large amounts of the purified tuberculin protein are used, animals can be sensitized to it in the same way as by infection with tubercle bacilli. Heidelberger & Menzel (9) isolated a series of fractions from the timothy-grass bacillus analogous to those previously obtained from *Streptococcus hemolyticus* by the same method.

Considerable interest has been shown in the heterogenetic antigens of bacteria. By absorption experiments Eisler (10) has found these to be different from the Forssman antigen in guinea-pig kidney and also to differ from one bacterial species to another. [See also Eisler & Howard (11) and Landsteiner & Levine (12).] On the other hand, Bailey & Shorb (13) found that the anti-sheep hemolysin produced by injecting Type I pneumococci into rabbits appeared to be the same as that induced by boiled sheep corpuscles. Moreover, rabbits immunized with sheep red cells were relatively resistant to infection with the pneumococcus. Further work is needed to clear up inconsistencies.

Ramon (14), in a discussion of the nature of diphtheria toxin, has concluded that all its properties can be explained on the basis of a single substance, rather than the complex mixture postulated by Ehrlich. Studies on the purification of this toxin by adsorption are grouped under (15); papers on the influence of the medium on the potency of the toxin under (15a), and those on the destructive or preservative action of various substances under (16). Moerch (17) believes that diphtheria toxin contains an unstable "flocculinogen," which influences the rapidity of flocculation of toxin-antitoxin mixtures and may occur in the filtrate from non-toxigenic strains. However, the experiments do not seem to have been controlled by dilutions with broth alone, which, according to Bunney, favorably influences the K_f. Hirsch (18) reports that the toxin extractable from dried,

defatted diphtheria cells appears identical with that in the broth and is given off to water at once, while the other nitrogenous extractives dissolve more slowly. The amount of toxin liberated increases with the age of the culture, as does also the amount of inorganic phosphorus, pointing to the enzymic degradation of a precursor.

For the influence of various substances on tetanus toxin see (19), on botulinus toxin (20), and on vibrio toxin (21).

Acetic-acid-precipitated staphylococcus toxin was shown by Burnet & Freeman (22) to be quite stable, especially at pH 7. Their best preparations contained 36,000 units per mg. of nitrogen. Formaldehyde detoxifies the toxin at a rate proportional to the concentration, the square root of the hydroxyl-ion concentration, and with a high temperature coefficient. In slow reactions in alkaline solutions an intermediate, reversible toxin-formaldehyde product is formed. Apparently only a single toxin is involved.

The action of formaldehyde on meningococcus toxin has been found by Klein (23) to be much like that on diphtheria toxin. Malcolm & B. White (24) have studied the endotoxin of meningococcus. It is antigenic and independent of the acetic acid-precipitable or heat-coagulable proteins of the micro-organism.

Presumably the type-specific antigen postulated by Enders as distinct from the specific polysaccharide (25) is being studied by Felton (26) in the form of an apparently protein- and carbohydrate-free product which contained nitrogen and protected mice type-specifically in doses as low as 0.004 mg. The homologous specific polysaccharide had an inhibiting effect.

The lysinogenic properties of bacteriophage have been found independent of biologically demonstrable bacterial protein by Bossa (27), and have also been studied by Kligler & Olitzki (28). Legroux & Djemil (29) pointed out similarities of phage to enzymes, and found it to be inactivated by formalin but still capable of giving rise to anti-phage in rabbits. Krueger (30) has published a critique of the serial-dilution method. An expression similar to one derived by Krueger for the kinetics of the binding of phage by bacteria has been found by Schlesinger (31) in an extensive investigation which includes the calculation of the particle size by two independent methods giving concordant results considerably at variance with those of ultrafiltration experiments.

Murphy and his collaborators (32) have found that the highly purified causative agent of a chicken tumor (33) gave rise only to

neutralizing antibodies. Analogies are pointed out between the tumor-producing agent and the "mutagen" of Pneumococcus, that is, the substance responsible for the change of an R-form into an S-form of the same or a different type (34).

2. Chemically Altered Proteins as Antigens

A study of the effect of alkali on the antigenic properties of proteins by Johnson & Wormall (35) disclosed that the precipitability of horse serum by antibody was markedly diminished at 19° and pH 12. If not too much alkali was used the treated serum could still be converted into an antigen by nitration, or by iodination—further evidence that the tyrosine groups in proteins play an important part in determining their antigenicity. Jacobs' (36) studies on iodinated sera as antigens showed that much weaker iodine solutions than Lugol's can cause the formation of iodo-antigen, pointing to the possible allergic origin of certain iodine disturbances in man.

Medveczky & Uhrovits (37) found that in benzoylating various sera and gelatin the products were soluble chemospecific antigens if the process was not pushed to the limit. Cross-reactions with other acyl proteins were observed; benzoyl gelatin (best containing 5 per cent of benzoyl) reacted to higher dilutions with the antisera than did the other proteins; benzoylated typhoid organisms gave the best antisera, and the precipitin reactions were not inhibited by sodium benzoate. Since the benzoylated antigens still reacted slightly with sera prepared from the native proteins, small amounts of unaltered protein might have been present. Formalin was found by Puccinnelli (38) to deprive eel and beef sera of their hemolytic properties with only a 50 per cent reduction in toxicity and no diminution in precipitinogenic power.

The distribution of intravenously injected antigen in rabbits was studied by Haurowitz & Breinl (39), using atoxylazo-horse serum. Most of the arsenic disappeared from the blood in six hours, at which time 25 to 30 per cent was recoverable from the liver and an equal amount from the marrow-containing bones. One-third of the arsenic was eliminated in the urine in 24 hours.

3. Haptens

a) General.—The relation between structure and specificity has been considered by Erlenmeyer & Berger (40) on the basis of

Grimm's theory of "*Feldwirkung*," or molecular fields (42). Because of the close serological relation existing between p-PhOC$_6$H$_4$NH$_2$, PhNHC$_6$H$_4$NH$_2$, and PhCH$_2$C$_6$H$_4$NH$_2$, when coupled by the diazo reaction with horse serum, it is concluded that haptens differing only in groupings whose fields of force are equal are indistinguishable by immunological means. The evidence presented loses in force because the technique used did not eliminate the possibility of cross-reactions due to unchanged horse serum, and since cross-reactions were obtained between PhCOC$_6$H$_4$NH$_2$ and the –NH and –O– haptens, although the force fields of the –CO– compound are quite different. In later work (41) the antimonic acid radical is shown to differ from the immunologically and chemically similar –PO$_3$H$_2$ and –AsO$_3$H$_2$ groups, and union of hapten and antibody *in vivo* is shown to occur.

Continuing their studies on conjugated carbohydrate proteins, Goebel, Babers & Avery (43) have synthesized the α- and β-p-aminophenol glucosides, coupled them with protein, and studied their immunological reactions [Avery, Goebel & Babers (44)]. While their previous work showed that β-glucosides of *different* sugars yielded rigidly specific antigens, it was now found that the α- and β-glucosides of the *same* sugar possessed antigenic properties in common, showing cross-precipitation in the antisera. However, in an α-glucoside horse-globulin antiserum, an excess of α-glucoside inhibited the specific reaction between antibody and α-glucoside chicken serum, while the β-glucoside did not. Similarly, in the β-antiserum the β-glucoside inhibited the homologous reaction, while the α-compound did not. These findings strikingly parallel the polysaccharide cross-reactions between Type II pneumococcus and the Type B Friedlander bacillus.

Landsteiner & van der Scheer (45) have again shown serological differences between steric isomers. These workers (46) have also prepared p-aminobenzoyl-glycylglycine and the similarly acylated glycyl-leucine, leucyl-glycine, and leucyl-leucine, and have coupled them with proteins. Each possessed a characteristic specificity, although there was some crossing between the glycyl-leucine and leucyl-leucine derivatives, indicating that the specificity depended more on the structure of the terminal amino acid carrying the free COOH group than on the other component of the dipeptide. Analogies with enzyme action are pointed out. The same workers (47) have prepared haptens by coupling p-amino-succinanilic, -adipanilic, or suberanilic acids with tyrosine or resorcinol. These actually precipi-

tated antisera to the homologous azo protein. Suberanilic-azo-resor-
cinol, which contains two azo groups and a long aliphatic chain,
reacted at as high a dilution as 1 : 1,000,000.

b) Specific polysaccharides.—A study of physico-chemical prop-
erties in this group by Heidelberger & Kendall (48) was a prelimi-
nary to attempts to determine molecular weights by the viscosity and
diffusion methods (49). The salts of the specific polysaccharide of
Type III pneumococcus (S III) are highly ionized and exhibit
anomalies in viscosity and diffusion of very great magnitude, owing
to the strongly acid nature and peculiar structure of the multivalent
anion. These anomalous viscosity effects are smaller in other, more
weakly acidic, specific carbohydrates. Corrections applied to the
data of Babers & Goebel (50) on the molecular weight of S III
changed their value from 118,000 to 2,400. A method was developed,
applicable to any specific polysaccharide, for the quantitative deter-
mination of S III, based on the amount of nitrogen specifically pre-
cipitated from an antibody solution calibrated with known amounts
of S III in the region of excess antibody (51). With the aid of this
method a tentative formula weight of 5,600 was assigned to S III,
and it was concluded that the formula weights of the specific poly-
saccharides as a group do not exceed 10,000.

Goodner, Dubos & Avery (52) have used the S III–splitting en-
zyme in rabbits, and found that the animals may be cured of an
otherwise fatal intradermal infection with Type III pneumococcus
if sufficient enzyme is given intravenously. Dubos (53) has studied
the factors affecting the yield of enzyme, finding that it is produced
by the soil bacillus only when S III is present in the medium, that
yeast extract is the best adjuvant, and that an accompanying toxic
product may be adsorbed by means of Type "C" aluminium hydrox-
ide at pH 5.5. Ward (54) has given evidence of a possible unstable,
highly antibactericidal intermediate product between the S III antigen
of the intact pneumococcus cell and the S III as isolated.

Specific carbohydrates of the typhoid-paratyphoid group have
been found by Meisel & Mikulaszek (55) to be distributed in much
the same way as the heat-stable agglutinogen. The S- and O-poly-
saccharides appeared identical, the R- and S-products different.
Tomcsik & Szongott (56) failed to find a relation between specific
polysaccharide content and virulence or presence of a capsule in
anthrax bacilli. However, the encapsulated organisms gave more of
a nitrogen-containing fraction, much of which was precipitable by

copper sulfate and was therefore unwarrantedly considered to be protein. Unfortunately, in both of these studies the specific polysaccharide agar was used as a culture medium and therefore presumably contaminated the products isolated.

In the tubercle bacillus group, McAlpine & Masucci (57) found the polysaccharide in unheated tuberculin from a human strain to be very sensitive to acid, even at ordinary temperatures. The d-arabinose component is considered the portion conferring reactivity with serum. Masucci, McAlpine & Glenn (58) found the polysaccharide in tuberculin from a bovine strain to contain no pentose and to show little cross-reaction with antibodies to the human type polysaccharide, differing in both respects from the main cellular carbohydrate fraction of the same bovine strain.[1] Heidelberger & Menzel (59) have isolated two independently specific polysaccharides from the complex carbohydrate mixture in the human type tubercle-bacillus cell, one of which, at any rate, seems also to occur in the bovine, avian, and timothy-grass types. The fractions are characterized by differences in optical rotation and in their ease of hydrolysis. Both carbohydrates occur in the human type tuberculin.[2] Gough (60) has attempted to identify the acid products of hydrolysis of the specific carbohydrate material and concluded that an acid similar to glycollic acid was present. H. du Mont & Anderson (61) removed all of the phosphorus from their specific polysaccharide material by purification over the acetyl derivative.

Morgan (62) has further described the specific carbohydrate of *B. dysenteriae* Shiga as a weak acid with $[\alpha]_{Hg\ green} = +107°$, a specific reactivity as high as $1:12,000,000$, and a nitrogen content of 1.8 per cent. Galactose and an acetylaminohexose are obtained on hydrolysis. The specific polysaccharide could be determined with a fair degree of accuracy by comparison of an unknown with the minimum amount necessary to kill passively sensitized guinea pigs.

Sordelli & Mayer (63) have confirmed their earlier work on cross-precipitin reactions with agar and have shown that the precipitins to it in anti-anthrax serum prepared from agar-grown organisms are independent of the true anthrax precipitins. Zozaya (64) has found that dextran, a simple polyglucose produced by *Leuconostoc mesenteroides*, gives precipitates in many bacterial antisera, but

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[1] Unpublished results by the reviewer and A. E. O. Menzel.

[2] *Ibid.*

that homologous antibodies are not absorbed. With Wood (65) he has recorded other cross-reactions, as has Savino (66) with a specific polysaccharide from *Staphylococcus aureus* grown in peptone-glucose broth.

The possible antigenic function of specific polysaccharides has again been studied. Zozaya (67) has reported that approximately 0.03 mg. of anthrax polysaccharide (containing agar?) adsorbed on collodion particles can produce precipitins in rabbits. Similar results were obtained with specific polysaccharides of other organisms, but no data are given on the purity of the preparations used. Pneumococcus polysaccharides did not react,[a] except in a horse. Casein and aluminium hydroxide particles were also effective carriers, but the polysaccharides alone, in 1 : 10,000 solution, failed to induce antibody formation. Zozaya explains the failure of collodion particles coated with immune serum to agglutinate in homologous polysaccharide solutions on colloid-chemical grounds. The reviewer believes agglutination fails owing to the soluble compound formed by the relative excess of polysaccharide much as in the agglutination inhibition discussed by Francis (see below).

That the heterogenetic hapten or haptens belong to the polysaccharide group rather than to the lipoids now seems increasingly evident (10, 11, 12).

c) Lipoids.—Additional experiments on highly purified lipoids have at last been carried out [cf. also (68)]. Weil & Besser (69) have shown that antibodies to cholesterol (*a*)–pig serum fail to react with dihydrocholesterol (*b*), or cholesterol dibromide, oxide, acetate, or palmitate. Antibodies could not be produced with the last four, but (*b*) yielded antisera of high specificity. The conclusion was reached (70) that chemically defined lipoids are comparatively nonreactive as antigens. An investigation by Berger & Scholer (71) included: (*a*); (*b*); ergosterol (*c*); irradiated ergosterol (*d*); crystalline vitamin D_2 (*e*); phytosterol; sitosterol (*f*); and analogous substances. Alcoholic solutions were mixed with pig serum for the injections, and great care was taken in controlling as many difficulties as possible. A very low percentage of rabbits responded with antibody formation; those injected with (*e*) died of intoxication; 8 of 17 on (*a*) died of anaphylactic shock, although no (*c*) rabbit died. The

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[a] S II and S III were not adsorbed in detectable amounts by collodion particles in experiments made by the reviewer.

lipoid-antibody content roughly paralleled the pig-serum-antibody titer. Flocculation reactions could not be obtained. An antiserum to (c) fixed complement only with c, d [which still contained (c)], and (f); but (c) reacted only with homologous antiserum. Antisera to (a) reacted only with (a), (b) (contrary to Weil & Besser), and in one case, with (d). It is concluded that pure lipoids may, through the "combination" method, stimulate the formation of antibodies with a high degree of specificity. The broad range of reactivity of alcoholic organ extracts would thus be due to the complexity of the mixtures used.

Work on the chemical nature of the antigens (haptens) of alcoholic brain extracts has been done by Rudy (72), who has also separated mixtures of lipoid haptens by differential adsorption (73). Plaut & Rudy (74) have made lengthy studies of the "masking" by serum lipoids of the power of alcoholic brain extracts to fix complement with antisera. The reviewer believes that the effect may be simply explained as an inhibition of the not very specific reaction by the excess of non-specific lipoids normally present in serum. The "masking" effect of lecithin on complement fixation by cholesterol-anti-cholesterol was also studied. The broad specificity of the serum lipoids of numerous animals was noted by Ishikawa (75). Gonzales, Armangué & Morato (76) found that while lipoids *per se* did not stimulate antibody production, adsorption on animal charcoal rendered them antigenic. These workers consider lipoids to be true antigens when present in the proper physical state. The antigenic properties of the floccules in human Wassermann-positive sera are discussed by Eagle (77).

A preliminary study of isohemagglutinogens has led Schröder (78) to consider them as lecithin analogs. These may easily be impurities, however, especially since Landsteiner (79), as well as Freudenberg and Brahn and their co-workers (79) believe specific carbohydrates to be involved.

4. Antibodies

The normal agglutinins for many bacteria in blood and urine may be concentrated 30,000-fold, according to Freund & Katz (80), by adsorption and elution, and mice may be protected against infection by small amounts of the concentrate.

In addition to speculations on the mode of formation of anti-

bodies (81) more evidence has appeared against the actual occurrence of antigen fragments in antibody. Berger & Erlenmeyer (82) failed to find arsenic in as much as 30 cc. of antiserum to atoxylazo-horse serum. An excellent review and discussion are given. Similar results were obtained by Hooker & Boyd (83) with atoxylazo-casein estimated to contain about 100 As groups in the molecule. Sox & Manwaring (84) interpret a series of experiments as a test-tube synthesis of new, intermediary, or hybrid specificities. Velluz (85) reports himself unable to confirm Salkowski on protein-free antitoxin, and A. Schmidt & Tuljtschinskaja (86) have found typhoid agglutinins to be almost completely destroyed even by a 1 to 25 dilution of active gastric juice in 2 hours at 37°.

Reiner & Reiner (87) have found in normal horse serum, fractions which are not specifically reactive, but are similar to those encountered in the purification of anti-pneumococcus serum by Felton. This worker (88) has been able to dissociate a portion of the specific precipitate of Pneumococcus I and II polysaccharides and antibody by solution in lime-water and addition of phosphate and calcium chloride to adsorb the polysaccharide. Felton has also further purified the water-insoluble pneumococcus antibody protein by removal of an inactive fraction with $AlCl_3$ at pH 5.2, or with $ZnCl_2$ at pH 7. The metal salt of the protein in the supernatant liquid was completely precipitable by specific polysaccharide, but only 80 to 90 per cent precipitated when freed from metal with carbonate or phosphate. The same was true of antibody protein recovered by dissociation. Pure antibody in amounts sufficient for chemical characterization would therefore seem almost at hand.

References to the preservation and stability of antibodies are grouped under (89). References to the distribution of antiviral and other antibodies among the serum fractions are given under (90). The stimulating action of tapioca on the production of precipitins has been shown by Liggeri (91), and of tapioca and calcium chloride on antitoxin production by Ramon & Lemétayer (92), although there is no agreement as to the mechanism involved. Hektoen & Delves (93) have investigated the relative independence of precipitins in polyvalent antisera.

In standardizing Type I anti-pneumococcus serum, W. Smith (94) has found a modification of the Dean-Webb optimal-proportions flocculation test to give a high degree of correlation with mouse protection, but to yield low values in the case of antibody solutions. The

quickest flocculation occurred at the equivalence point.[4] Duplicate quantitative analyses of specifically precipitable nitrogen, as proposed by Heidelberger, Sia, & Kendall (95), are avoided but approximately one hour and twenty minutes of manipulation are required for each serum,[5] while the analytical method requires only 40 minutes of manipulation in the case of a single serum and 25 minutes for each additional serum analyzed at the same time. Zozaya (96) has applied his method of standardization, also based on precipitation with specific polysaccharide, to anti-dysentery Shiga serum and to anti-meningococcus serum. A dilution method is used by Glenny & Barr (97) in comparing the avidities of antitoxins. [See also Moerch (17 and 98) on this point.] Electrodialysis is said by Gerlough & W. White (99) greatly to improve the stability of diphtheria- and tetanus-antitoxin concentrates, removing a small fraction rich in lipoid and phosphorus.

II. THE CHEMISTRY OF IMMUNE REACTIONS

a) *General.*—Sachs & Behrens (100) report a series of complex "model" experiments, many employing tannin, which are interpreted as failing to confirm the theory of Stern and work of Reiner and of Freund, that the action of antibody on antigen is one of dehydration.

Marrack & Smith (101) have confirmed the chemical combination of hapten with antibody by dialyzing uncombined hapten—in this case atoxylazotyrosine—from mixtures with normal globulin and with antibody to atoxylazo-horse globulin, finding in the latter case up to a twenty-fold increase in the ratio of bound to free hapten. Adsorption was ruled out since the antibody failed to bind more of the hapten-like methyl red than did normal globulin. Failure to consider the fundamental chemical union between hapten and antibody has led Ward (102) into error in discussing the effect of Type I pneumococcus antiserum in pneumonia. A dose of 200 cc. of serum would neutralize the amount of S I in only about 500 cc. of culture, not 12,500 liters, as calculated by Ward.

b) *The precipitin reaction.*—The use of this reaction in the quantitative estimation of a specific polysaccharide (51) and in the stand-

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[4] This term is suggested by the reviewer as preferable to "equilibrium point" or "neutralization point."

[5] Reviewer's calculation.

ardization of antisera (94, 96) has been mentioned. Culbertson & Seegal (103) have employed it for the determination of antibody in anti-egg albumin serum. Applying Heidelberger, Sia & Kendall's method (95) it was found that the ratio of egg albumin to antibody precipitated at the "neutralization point" (at which neither antigen nor antibody could be demonstrated in the supernatant liquid) was 1 : 13. A series of small tests is set up with increasing amounts of a solution of crystalline egg albumin, of which mg.-at-the-"neutralization-point" $\times$ 13 $=$ antibody protein. A similar method, based on the Dean-Webb optimal-proportions procedure, has been advanced by Taylor, Adair & Adair (104) for the estimation of proteins.[6] Crystalline egg albumin and horse globulin were used. Culbertson (105) has been able to determine the blood volume of rabbits by the precipitation method. Masuda (106) has studied the speed of formation of the specific precipitate, as has also Eagle (107) in the course of a study of the factors influencing the rate of immune aggregation reactions in general. The results are considered to support Eagle's theory of the mechanism of aggregation reactions, but since the effects are in the direction demanded by any simple chemical reaction, their bearing on the theory is not clear. The influence of salts on the precipitin reaction has been studied by Downs & Gottlieb (108).

c) *Agglutination.*—Francis (109), studying the effect of specific polysaccharide on agglutination of pneumococci, has found an extensive parallel with the precipitin reaction and concludes that both reactions are expressions of the same chemical mechanism. Bier (110) has found the "agglutination optimum" to shift to lower serum dilutions with increasing concentration of salt.

d) *Anaphylaxis.*—Neill, Sugg & Richardson (111) have passively sensitized guinea pigs with diphtheria antitoxin and shocked them with toxin, considering the effect as a typical anaphylactic shock produced by the toxin itself and not by nucleoprotein or polysaccharide. Meyers (112) has shown that fibrinogen is absent in many antibody concentrates capable of causing serum disease, so that it cannot be the causative factor. Gebauer-Fuelnegg, Dragstedt & Mullenix (113) have found in the lymph and blood of dogs during anaphylactic shock a dialyzable substance giving the reactions of histamine.

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[6] The method given in (51) is also applicable to proteins.

e) *Hemolysis.*—H. von Euler & Gard (114) found the normal hemolytic power of sheep serum toward rabbit cells to be due to a typical "amboceptor" (*A*) and a second component, apparently the mid-piece of sheep complement. H. von Euler & Brunius (115) have studied the effect of pH and salt concentration on the union of red-cell stromata and lipoids with (*A*), as well as of the elution of (*A*) from the complex at alkaline reactions. The speed of the reaction was studied, also the amount of hemolysis in constant time at different (*A*) concentrations. Ponder (116) has continued his work on the kinetics of hemolysis, introducing correction terms for the velocity of mixing and for the inhibition of hemolysis by the hemoglobin liberated. The experimental procedure and reasoning are difficult and involved, although orderly and apparently reasonable if the assumptions made are granted. It was found, under the conditions used, that in the lysis of a given number of cells a constant amount of complement is used up irrespective of the amount of (*A*) used for sensitization. Olitzki (117) observed that silica gel removes anticomplementary material from serum and may be used for the adsorption of antibodies.

f) *The toxin-antitoxin reaction.*—H. Schmidt, Scholz & Perry (118) have made quantitative studies on the dissociation of the diphtheria and tetanus toxin-antitoxin complexes by formalinized toxin. The evidence is considered in favor of the Bordet adsorption theory, but the arbitrary conditions chosen raise doubt as to whether equilibrium was established. On this work H. Schmidt & Scholz (119) have based a method for the evaluation of formol toxoids, as well as the opinion that toxin possesses a greater affinity for antitoxin than does toxoid, a conclusion exactly the opposite to that reached by S. Schmidt and Ramon from a study of the same reaction. The situation thus supports the reasoning of Heidelberger & Kendall that the findings are characteristic of a true chemical equilibrium in which affinity relations affect only the extent of dissociation.[7] Hansen (120) has modified Ramon, Legroux & Schoen's method of dissociating the diphtheria anatoxin-antitoxin precipitate by heat. Fifteen minutes in boiling water containing 2.4 per cent of $Na_2HPO_4 \cdot 12H_2O$ sufficed to yield 50 to 90 per cent of the anatoxin of a 200–300-fold degree of purity. This could be increased to 400-fold by adsorption on alumina followed by elution. The prod-

[7] Cf. *Ann. Rev. Biochem.,* 1, 669.

uct, however, still contained enough horse-serum protein to shock sensitized guinea pigs.

The reaction between staphylococcus toxin and antitoxin has been analyzed further by Burnet (121). At the flocculation optimum an excess of antitoxin is present. The quantitative data presented are considered best explained by adsorption of excess antitoxin by the precipitate, although in the case of the anatoxin it is considered that combination may occur in the proportions TA and T_2A.

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PRESBYTERIAN HOSPITAL
620 WEST 168TH STREET
    NEW YORK CITY